REMARKS

Amendments

Claim 1 has been amended to more clearly claim the subject matter of the present invention. Claim 1 now specifies that the expression construct comprises the adenoviral VA1 gene and a nucleic acid encoding an RNAi molecule in which the RNAi molecule is defined as a substrate for Dicer and in which the nucleic acid encodes an shRNA or an miRNA. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid construct is inserted within the adenoviral VA coding sequence. The RNAi molecule is a substrate for Dicer upon expression. Support for these amendments can be found in Figures 1A and 1B, Figures 2A and 2C, Figure 3 and paragraphs [0018]-[0023], [0027], [0028] and [0031] of the published application.

Claim 2 has been amended to be consistent with claim 1 as amended.

Claim 5 has been amended to be consistent with claim 1 as amended and to delete the term "about."

Claim 6 has been amended to delete the term "about."

Claim 11 has been amended in the similar manner as claim 1 and finds support in the same portions of the application.

Claims 13 and 14 have been amended to be consistent with claim 1 as amended.

Claims 15 and 16 have been amended to be consistent with claim 11 as amended.

It is submitted that these amendments do not constitute new matter, and their entry is requested.

Rejection Under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claim 5 under 35 U.S.C. § 112, second paragraph for being indefinite. Although Applicants do not believe that the term "about" renders the claims indefinite to a person of skill in the art, they have nevertheless deleted this term from claims 5 and 6.

In view of the above amendments and remarks, Applicants submit that claim 5 is definite. Withdrawal of this objection is requested.

Rejection Under 35 U.S.C. § 102(e)

The Examiner has rejected claims 1, 11, 12, 13 and 15 under 35 U.S.C. § 102(e) as being anticipated by Agami et al. (US 7,241,618). Applicants traverse this rejection.

Agami et al. discloses a polynucleotide that comprises an RNA polymerase III promoter, a region encoding an siRNA and a transcriptional termination element. See, column 8, lines 47-51. According to column 9, lines 33-55, Agami discloses that the RNA polymerase III promoter are responsible for expression of a variety of genes including adenovirus VA1. According to column 9, line 65 - column 10, line 4, the promoter "will be operably linked" to the siRNA encoding region. The siRNA encoding region is typically "immediately downstream of the transcriptional start site or be separated by a minimal distance such [sic - as] less than twenty base pairs, preferably less than ten base pairs, even more preferably less than five base pairs and still more preferably by two or less base pairs." There is no disclosure in Agami et al. of an expression cassette comprising the adenoviral VA1 gene and a nucleic acid encoding an RNAi molecule. There is no disclosure in Agami et al. of the nucleic acid encoding RNAi molecule being inserted within the VA1 RNA coding sequence. Claims 1 and 11 have been amended to clearly define the nature of the expression cassette which is not disclosed in Agami et al. In view of these deficiencies in Agami et al., Applicants submit that Agami et al. does not anticipate the claimed subject matter. In addition, Applicants note that Agami et al. does not suggest the claimed subject matter.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not anticipated by Agami et al. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1 and 11-16 under 35 U.S.C. § 103(a) as being obvious over Agami et al. or Polo et al. (US 6,329,201) taken with either Yu et al. (*Proc Natl Acad Sci USA* **99**:6047-6052, 2002) or Ambros (*Cell* **107**:823-826, 2001). In essence, the Examiner cites (a) Polo et al. for its disclosure of an expression construct comprising an adenoviral VA1 promoter operatively linked to an antisense molecule, (b) Yu et al. for its disclosure of an RNA pol III vector comprising shRNA which can inhibit expression in mammalian cells and (c) Ambros for its disclosure of miRNA. Thus, he concludes that it would have been *prima facie* obvious to produce (i) an *in vitro* cell comprising an expression vector comprising an adenoviral VA1 promoter operatively linked to a construct comprising RNAi, (ii) an RNA polymerase III vector comprising shRNA and (iii) a vector encoding microRNA. Applicants traverse this rejection.

As described above with respect to the anticipation rejection, Applicants note that Agami et al. does not teach or suggest the expression cassette as set forth in the present claims. Similarly, Polo et al. does not describe or suggest the claimed expression cassette. Polo et al. teaches that in an alternative embodiment, the antisense sequence may be expressed under control of an RNA polymerase III promoter. See, column 31, lines 24-26. Polo et al. provides the adenovirus VA1 promoter as one example of an RNA polymerase III promoter. According to column 31, lines 26-33 describe a promoter construct that comprises the "Adenovirus 2 VA1 RNA promoter (nucleotides -70/+30), nucleotides 7562-7606 of Sindbis virus, and the RNA polymerase III consensus transcription termination sequence." As is evident from this passage, Polo et al. does not describe an expression cassette which comprises the adenoviral VA1 gene with the nucleic acid encoding the RNAi molecule inserted within the adenoviral VA1 coding sequence for the VA1 RNA.

Neither Yu et al. nor Ambros cure the deficiencies of Agami et al. or Polo et al. That is, neither Yu et al. nor Ambros describe the claimed expression cassette. Thus, Applicants submit that the claimed subject matter is not obvious from the cited references.

In view of the above amendments and remarks, Applicants submit that the combination of Agami et al. or Polo et al. taken with either Yu et al. or Ambros does not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1 and 2 under 35 U.S.C. § 103(a) as being obvious over Agami et al. or Polo et al. taken with either Yu et al. or Ambros further in view of Doglio et al. (US 5,837,503). The Examiner cites Agami et al., Polo et al., Yu et al. and Ambros as in the previous rejection, and cites Doglio et al. for its disclosure of an expression cassette in which an oligonucleotide (e.g., antisense or ribozyme) has been inserted between or outside the boxes A and B constituting the promoter of the VA1 gene. The Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette comprising an adenoviral VA promoter in which an RNAi molecule is contained within a non-essential stem region of the promoter. Applicants traverse this rejection.

It was well known at the time of filing the present application that shRNA is processed by Dicer to form siRNA of shorter length. See, paragraph 7 of the present application. For example, Bernstein et al. (*Nature* **409**:363-366, 2001; cited by the Examiner in the previous Office Action) teaches that the siRNAs have a length of 22 nucleotides. Similarly, Yu et al. teaches that the shRNA is processed by Dicer to form an siRNA of about 21 nucleotides. Thus, it was well known to a skilled artisan that it was necessary to process dsRNA, e.g., shRNA, to the shorter length siRNA in order to produce an RNAi molecule that had inhibitory properties on gene expression. Interference was not achieved without the cleavage of the shRNA by Dicer into the smaller siRNA.

As shown in Rossi et al. (US 6,100,087; cited by the Examiner in the previous Office Action) as well as Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000; cited by the Examiner in the present Office Action), it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. There is no disclosure in Rossi et al., or any of the other cited

art, to suggest that any RNA inserted into the VA1 transcript would be processed out of the VA1 transcript. For example, there is no disclosure in Doglio that the antisense RNA or ribozyme RNA would be processed out of the VA1 transcript. In fact, Rossi et al. teaches exactly the opposite effect, namely that an interfering RNA molecule (i.e., ribozyme) is not processed out of the transcript. Because the interfering RNA molecule (i.e., ribozyme) of Rossi et al. is not processed out of the VA1 transcript, there is no motivation to substitute a different interfering molecule, e.g., shRNA which must be processed out of the VA1 transcript and cleaved by Dicer in order to be active, for the ribozyme of Rossi et al.

Not only is there no motivation in the cited art to make such a substitution, but there is no suggestion in the art for a skilled artisan to reasonably expect that the shRNA inserted into the VA1 transcript would be cleaved out, particularly in view of the specific teachings of Rossi et al. and Cagnon et al., thus making it a substrate for Dicer. If the shRNA is not cleaved out of the VA1 transcript, the shRNA is not a substrate for Dicer. Figure 3 of the present application shows that the shRNA is cleaved out of the VA1 transcript and this shRNA is then a substrate for Dicer to form the active siRNA. This result is not expected from any of the teachings of the prior art. Claims 1 and 11 contain a specific limitation that the RNAi molecule is a substrate for Dicer. This limitation is not taught or suggested in any of the cited prior art references.

Furthermore, Applicants submit that the prior art, namely Rossi et al. and Cagnon et al., teaches away from the presently claimed subject matter and provides no motivation to make the combination proposed by the Examiner in view of this specific teaching away. Consequently, Applicants submit that the claimed subject matter is not obvious from the cited prior art.

In view of the above amendments and remarks, Applicants submit that the combination of Agami et al. or Polo et al. taken with either Yu et al. or Ambros further in view of Doglio et al. does not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 2 and 3 under 35 U.S.C. § 103(a) as being obvious over Agami et al. or Polo et al. taken with either Yu et al. or Ambros and Doglio et al. and in further view of Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000). The Examiner cites Agami et al., Polo et al., Yu et al., Ambros and Doglio as in the previous rejection and cites Cagnon et al. for its disclosure of inserting an RNAi molecule (i.e., ribozyme) using a filled in NotI site that was ligated into the BstEII cleaved, filled in vector. The Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette in which the non-essential region contains a BstEII site. Applicants traverse this rejection.

It was well known at the time of filing the present application that shRNA is processed by Dicer to form siRNA of shorter length. See, paragraph 7 of the present application. For example, Bernstein et al. (*Nature* **409**:363-366, 2001; cited by the Examiner in the previous Office Action) teaches that the siRNAs have a length of 22 nucleotides. Similarly, Yu et al. teaches that the shRNA is processed by Dicer to form an siRNA of about 21 nucleotides. Thus, it was well known to a skilled artisan that it was necessary to process dsRNA, e.g., shRNA, to the shorter length siRNA in order to produce an RNAi molecule that had inhibitory properties on gene expression. Interference was not achieved without the cleavage of the shRNA by Dicer into the smaller siRNA.

As shown in Rossi et al. (US 6,100,087; cited by the Examiner in the previous Office Action) as well as Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000; cited by the Examiner in the present Office Action), it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. There is no disclosure in Rossi et al., or any of the other cited art, to suggest that any RNA inserted into the VA1 transcript would be processed out of the VA1 transcript. For example, there is no disclosure in Doglio that the antisense RNA or ribozyme RNA would be processed out of the VA1 transcript. In fact, Rossi et al. teaches exactly the opposite effect, namely that an interfering RNA molecule (i.e., ribozyme) is not processed out of the transcript. Because the interfering RNA molecule (i.e., ribozyme) of Rossi et al. is not processed

out of the VA1 transcript, there is no motivation to substitute a different interfering molecule, e.g., shRNA which must be processed out of the VA1 transcript and cleaved by Dicer in order to be active, for the ribozyme of Rossi et al.

Not only is there no motivation in the cited art to make such a substitution, but there is no suggestion in the art for a skilled artisan to reasonably expect that the shRNA inserted into the VA1 transcript would be cleaved out, particularly in view of the specific teachings of Rossi et al. and Cagnon et al., thus making it a substrate for Dicer. If the shRNA is not cleaved out of the VA1 transcript, the shRNA is not a substrate for Dicer. Figure 3 of the present application shows that the shRNA is cleaved out of the VA1 transcript and this shRNA is then a substrate for Dicer to form the active siRNA. This result is not expected from any of the teachings of the prior art. Claims 1 and 11 contain a specific limitation that the RNAi molecule is a substrate for Dicer. This limitation is not taught or suggested in any of the cited prior art references.

Furthermore, Applicants submit that the prior art, namely Rossi et al. and Cagnon et al., teaches away from the presently claimed subject matter and provides no motivation to make the combination proposed by the Examiner in view of this specific teaching away. Consequently, Applicants submit that the claimed subject matter is not obvious from the cited prior art.

In view of the above amendments and remarks, Applicants submit that the combination of Agami et al. or Polo et al. taken with either Yu et al. or Ambros and Doglio et al. and in further view of Cagnon et al. does not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 5 and 6 under 35 U.S.C. § 103(a) as being obvious over Agami et al. or Polo et al. taken with either Yu et al. or Ambros and in further view of Lorens (US 2004/0005593). The Examiner cites Agami et al., Polo et al., Yu et al. and Ambros as in the initial obviousness rejection and cites Lorens for its disclosure of an RNAi molecule having a loop

containing at least 6 nucleotides. The Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette comprising an adenoviral VA promoter in which an RNAi molecule comprises a loop containing about 8 nucleotides. Applicants traverse this rejection.

It was well known at the time of filing the present application that shRNA is processed by Dicer to form siRNA of shorter length. See, paragraph 7 of the present application. For example, Bernstein et al. (*Nature* **409**:363-366, 2001; cited by the Examiner in the previous Office Action) teaches that the siRNAs have a length of 22 nucleotides. Similarly, Yu et al. teaches that the shRNA is processed by Dicer to form an siRNA of about 21 nucleotides. Thus, it was well known to a skilled artisan that it was necessary to process dsRNA, e.g., shRNA, to the shorter length siRNA in order to produce an RNAi molecule that had inhibitory properties on gene expression. Interference was not achieved without the cleavage of the shRNA by Dicer into the smaller siRNA.

As shown in Rossi et al. (US 6,100,087; cited by the Examiner in the previous Office Action) as well as Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000; cited by the Examiner in the present Office Action), it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. There is no disclosure in Rossi et al., or any of the other cited art, to suggest that any RNA inserted into the VA1 transcript would be processed out of the VA1 transcript. For example, there is no disclosure in Doglio that the antisense RNA or ribozyme RNA would be processed out of the VA1 transcript. In fact, Rossi et al. teaches exactly the opposite effect, namely that an interfering RNA molecule (i.e., ribozyme) is not processed out of the transcript. Because the interfering RNA molecule (i.e., ribozyme) of Rossi et al. is not processed out of the VA1 transcript, there is no motivation to substitute a different interfering molecule, e.g., shRNA which must be processed out of the VA1 transcript and cleaved by Dicer in order to be active, for the ribozyme of Rossi et al.

Not only is there no motivation in the cited art to make such a substitution, but there is no suggestion in the art for a skilled artisan to reasonably expect that the shRNA inserted into the VA1 transcript would be cleaved out, particularly in view of the specific teachings of Rossi et al. and

Cagnon et al., thus making it a substrate for Dicer. If the shRNA is not cleaved out of the VA1 transcript, the shRNA is not a substrate for Dicer. Figure 3 of the present application shows that the shRNA is cleaved out of the VA1 transcript and this shRNA is then a substrate for Dicer to form the active siRNA. This result is not expected from any of the teachings of the prior art. Claims 1 and 11 contain a specific limitation that the RNAi molecule is a substrate for Dicer. This limitation is not taught or suggested in any of the cited prior art references.

Furthermore, Applicants submit that the prior art, namely Rossi et al. and Cagnon et al., teaches away from the presently claimed subject matter and provides no motivation to make the combination proposed by the Examiner in view of this specific teaching away. Consequently, Applicants submit that the claimed subject matter is not obvious from the cited prior art.

In view of the above amendments and remarks, Applicants submit that the combination of Agami et al. or Polo et al. taken with either Yu et al. or Ambros and in further view of Lorens does not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Rejection for Obviousness-type Double Patenting

The Examiner has rejected claims 1 and 11-16 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) with Frey et al. (actually Benn and Frey, Abstracts of General Meeting of American Society of Microbiology, 92:225, H254, 1992) in view of Zeng et al. (*Mol Cell* **9**:1327-33, 2002). . In his reasons for this rejection, the Examiner further cites Yu et al. Applicants traverse this rejection.

Rossi et al. discloses an expression cassette that includes the U16 snoRNA and, for example, a hammerhead ribozyme specific to HIV-1 RNA and a TAR element. This construct results in the localization of the expression product to the nucleoli where it inhibits HIV replication. There is no disclosure in Rossi et al. of a nucleic acid encoding an RNAi molecule that is a substrate for Dice that is inserted within the coding sequence of the adenoviral VA1 gene. Because the purpose of Rossi et al. was to achieve localization of the expression product in the nucleoli, there would be no

motivation to modify the expression cassette in a manner that would lead to cellular localization and not nucleoli localization. It was well known at the time of Benn and Frey that the adenoviral VA1 gene localized to the cytoplasm. There is no teaching in either Zeng et al. or Yu et al. that would provide any motivation for modifying Rossi et al. as proposed by the Examiner. Thus, the skilled artisan would not have combined the cited references in the manner proposed by the Examiner in making this rejection. Consequently, Applicants submit that the claimed subject matter is not obvious from the cited references. Since the claimed subject matter is not obvious from the cited references, Applicants submit that the instant obviousness-type double patenting rejection is improper.

In view of the above remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al., Benn and Frey and Zeng et al. Withdrawal of this rejection is requested.

Rejection for Obviousness-type Double Patenting

The Examiner has rejected claims 1, 5 and 6 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) with Frey et al. (actually Benn and Frey) in view of Zeng et al. In his reasons for this rejection, the Examiner further cites Yu et al. and Lorens, neither of which provide any motivation for modifying Rossi et al. in the manner proposed by the Examiner. Applicants traverse this rejection for the same reasons as the previous obviousness-type double patenting rejection.

In view of the above remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al., Benn and Frey and Zeng et al. Withdrawal of this rejection is requested.

Rejection for Obviousness-type Double Patenting

The Examiner has rejected claims 1, 2 and 3 for obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) with Frey et al. in view of Zeng et al. In his reasons for this rejection, the Examiner further cites Yu et al. and Cagnon et al., neither of which provide any motivation for modifying Rossi et al. in the manner proposed by the Examiner. Applicants traverse this rejection for the same reasons as the previous obviousness-type double patenting rejections.

In view of the above remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al., Benn and Frey and Zeng et al. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, Applicants believe that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application..

Respectfully submitted,

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